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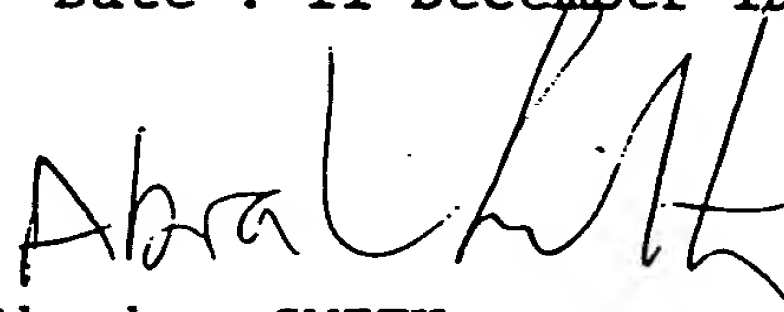
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VACCINE AGAINST INFECTIOUS AGENTS HAVING AN INTRACELLULAR PHASE, COMPOSITION FOR THE TREATMENT AND PREVENTION OF HIV INFECTIONS, ANTIBODIES AND METHOD OF DIAGNOSIS.

*Jual*  
*OTDI*  
5 The present invention relates to new types of vaccines and, in particular, to compositions intended for the treatment, prevention and diagnosis of HIV conditions.

10 More specifically, the present invention relates to peptides capable of producing an immune response capable of directly or indirectly neutralizing HIV viruses in mammals and in particular in man.

15 The importance of monoclonal antibodies directed against  $\beta_2$ -microglobulin ( $\beta_2m$ ) in the inhibition of HIV-1 replication has already been described, particularly in patent EP-B-0,470,989 as well as in various publications.

In particular, it has been possible to demonstrate that these antibodies act on two mechanisms, namely directly on the virus and on the cells associated with  $\beta_2m$ .

20 The present invention constitutes developments of these preliminary elements and is based on the identification of peptide sequences obtained from  $\beta_2m$  or having an equivalent structure which are capable of generating antibodies completely or partially neutralizing the HIV  
25 viruses.

Given the complexity of the mechanisms used, "neutralization of the HIV virus" will be understood to mean any mechanism having the effect in vivo of destroying and/or of preventing the propagation of viruses.

30 In vitro, these neutralizing antibodies can be used to neutralize any body fluid intended to be reinoculated or reintroduced into man, such as the sperm of a man seropositive for HIV for the insemination of a seronegative woman.

35 However, more generally, the present invention is based on a new vaccinal approach which can be used, in particular, for infectious agents of the parasite or virus type with a high mutating power. Indeed, in the context of traditional vaccination, it is sought to

generate neutralizing antibodies directed against components of the infectious agent, but when the latter exhibits a high mutating power, such as HIV for example, this strategy gives, at best, only limited results for a particular isolate which will be very rapidly replaced by a mutant and resistant isolate.

The new vaccinal approach is based on a different concept and is applicable to a number of infectious agents which have an intracellular phase during their cycle.

Indeed, it is known for certain agents, or it is possible to demonstrate, especially in the case of HIV, which constitutes part of the present invention, that, during the multiplication of the infectious agents from the infected cells of the host, the extracellular infectious agents carry away components of determinants of the host cell.

One of the subjects of the present invention consists in taking as target, not the infectious agent itself, but the components of the determinant which it carries away with it and to provide for a vaccination directed against these cellular determinants which will remain constant, even if the agent itself has mutated.

This type of approach has, of course, an immediate limit, the antigen being bound to the host cells, it is only possible to carry out such a vaccination with a cryptic epitope of the cellular determinant which will be exposed only when it is carried away by the extracellular infectious agent, or an epitope which is nonimmunogenic in its natural presentation by the cell and which is modified when it is presented at the surface of the virion.

In the case of HIV for example, it has been possible to demonstrate that  $\beta_2$ -microglobulin has several cryptic epitopes, which are exposed during the multiplication of the HIV virus and its passage outside the cell. There is not therefore, in the event of vaccination, on the one hand, an autoimmune reaction, and, on the other hand, the epitope being bound to the different HIV

isolates which have been tested, the vaccination is effective, this being independent of the mutations of the virus itself.

5 This type of vaccination can be selected, in particular, for intracellular parasites and enveloped viruses such as CMV, HPV, HSV and HIV for example.

10 It should be clearly understood that while this type of vaccination cannot be used in all cases, it can constitute a very useful alternative for infectious agents which are resistant to more traditional approaches.

15 Accordingly, the present invention relates to a vaccine against an infectious agent, characterized in that it comprises at least one cryptic epitope of a cellular element carried away by an intracellular infectious agent during its passage outside the cell and which is exposed by the infectious agent.

20 Preferably, this infectious agent is a parasite or an envelope virus and the cryptic epitope is situated near the surface of the cell.

25 "Cryptic epitope" is intended to designate an epitope of a cellular determinant of the host which is hidden or modified and is therefore recognized as being foreign by the immune system and does not therefore produce an autoimmune reaction with destruction of the corresponding determinant and which can be used for vaccination.

30 The cryptic epitope should obviously be exposed, that is to say be accessible and recognized by the immune system when it is carried away by the infectious agent (in the event that it should remain cryptic, the vaccination would not be possible).

35 In the case of  $\beta_2$ -microglobulin, it has been possible to demonstrate the existence of this type of epitope which is in fact also found in a natural form during the elimination of  $\beta_2$ -microglobulin by the urinary tract.

The present invention therefore relates to compositions intended for the treatment or prevention of

HIV infections, characterized in that they comprise, as active ingredient, at least one peptide corresponding to sequences 1 to 22 or an equivalent sequence. "Equivalent sequence" is intended to designate a sequence which lifts the neutralization of the HIV virus by the monoclonal antibodies B1G6 or B262.2 in vitro.

These peptides constitute cryptic epitopes of  $\beta_2$ -microglobulin as described above.

*Sub B1* The peptides according to the present invention are the following:

01-P1 IQRTPKIQVYSRHPA

(Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr-Ser-Arg-His-Pro-Ala)

02-P4 FHPSDIEVDLLKDGE

15 (Phe-His-Pro-Ser-Asp-Ile-Glu-Val-Asp-Leu-Leu-Lys-Asp-Gly-Glu)

03-P9 ACRVNHVTL SQPKIV

(Ala-Cys-Arg-Val-Asn-His-Val-Thr-Leu-Ser-Gln-Pro-Lys-Ile-Val)

20 It is also possible to use a smaller part (7 amino acids) of these 15 amino acids which lifts the neutralization of the virus by the monoclonal antibodies B1G6 or B2G2.2:

04-R-7-V RTPKIQV (Arg-Thr-Pro-Lys-Ile-Gln-Val)

25 05-S-7-K SQPKIVK (Ser-Gln-Pro-Lys-Ile-Val-Lys)

06-F-7-E FHPSDIE (Phe-His-Pro-Ser-Asp-Ile-Glu)

*Sub B2* A common structure PKI (3 amino acids) appears to be the unit which is responsible; hence the following amino acid modifications:

30 07-TLSRTPKIQV (Thr-Leu-Ser-Arg-Thr-Pro-Lys-Ile-Gln-Val)  
No. 185

08-IYLTQPKIKV (Ile-Tyr-Leu-Thr-Gln-Pro-Lys-Ile-Lys-Val)  
No. 186

35 09-IQRTPKIQVY (Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr)  
No. 187

10-TLSQPKIVKN (Thr-Leu-Ser-Gln-Pro-Lys-Ile-Val-Lys-Asn)  
No. 188

11-IQRTPOIVKW (Ile-Gln-Arg-Thr-Pro-Gln-Ile-Val-Lys-Trp)  
No. 189



12-IQRTPNIVKW (Ile-Gln-Arg-Thr-Pro-Asn-Ile-Val-Lys-Trp)  
No. 190

*Sub 3* It is also possible to introduce a cysteine and a glycosylation site:

13-CYNPSDIE (Cys-Tyr-Asn-Pro-Ser-Asp-Ile-Glu)

14-YCNPEST (Tyr-Cys-Asn-Pro-Glu-Ser-Thr)

15-NFLNCYVS (Asn-Phe-Leu-Asn-Cys-Tyr-Val-Ser)

16-LNCYVSPSD (Leu-Asn-Cys-Tyr-Val-Ser-Pro-Ser-Asp)

Finally, it is possible to use the peptides using the different variations according to the species (mice, primates, rabbits, guinea pigs):

17-KTPQIQV (Lys-Thr-Pro-Gln-Ile-Gln-Val)

18-FHPPQIE (Phe-His-Pro-Pro-Gln-Ile-Glu)

19-FHPPHIE (Phe-His-Pro-Pro-His-Ile-Glu)

20-AEPKTVY (Ala-Glu-Pro-Lys-Thr-Val-Tyr)

21-SQPKTVY (Ser-Gln-Pro-Lys-Thr-Val-Tyr)

22-ILSRTPKIQV (Ile-Leu-Ser-Arg-Thr-Pro-Lys-Ile-Gln-Val)

These peptides of SEQ ID 1 to 22 contain only the preferential choice; it is possible, as has been indicated above, to find equivalent peptides.

Example 5 describes a method which makes it possible to identify the equivalent peptides.

These peptides are preferably bound to a carrier system; this may be either one or more protein fragments linked to the N- and/or C-terminal ends of said peptides in order to allow, in particular, an immune response; they will then be referred to as "conjugated proteins". Among the proteins which can be used, there may be mentioned in particular albumins, KLH (Keyhole Limpet Hemocyanin) MAP (Multiple Antigenic Peptide) or other proteins known for their immunogenicity. It is also possible to envisage proteins or protein fragments linked through nonpeptide bonds such as a disulfide bridge or bonds through a calcium ion.

During the study of the various peptides according to the invention, it emerged, although this is only a theory which cannot limit the present invention in any manner, that the PKI structure plays an essential role. Indeed, proline is an amino acid which imposes a confor-

mation and which limits the possibility of a quaternary peptide configuration. Under these conditions, KI (Lys, Ile) is attached in a position which is exposed to reacting with an antibody.

5 Under these conditions, during the construction of the carrier proteins, it is advisable to provide preferably for a structure which leaves the PKI structure accessible.

*Sub B4*  
10 Analysis of the structure of the regions selected for P1, P9 and P10 can be carried out by methods such as the selection using alanine to replace each amino acid separately, particularly in the RTPKIQV region, in order to determine the possible amino acids. It is also possible to use techniques using biotinylation of each  
15 peptide, followed by selection by EIA with the antibodies in order to determine the loss of attachment.

It is thus possible to envisage conjugating the epitopes in question with nonprotein components, for example polysaccharides and/or lipids, in order to  
20 constitute lipoproteins having enhanced vaccinating activities; here again, it is possible to envisage covalent bonds or otherwise.

These various types of compounds can be obtained either by chemical synthesis or by recombinant routes  
25 using techniques known in the field of production of recombinant proteins.

The flexibility of recombinant technologies makes it possible to produce proteins having a plurality of identical or different epitopes and capable of enhancing  
30 the activity of the final product. It is also possible to envisage the co-expression of various components entering into the compositions according to the invention.

According to one of the aspects of the invention, it will be possible for the peptide to be introduced into  
35 a known structural protein of HIV; in particular, constructs in which the peptide of interest is inserted into the hypervariable region of the V3 loop of gp120 can be used.

The V3 region of gp120 is the principal HIV-1



neutralization domain and one of the major determinants of viral tropism. Consequently, this type of mutant can be useful for studying the neutralization of HIV-1 linked to R7V and the modifications of its host spectrum. The high variability of the V3 region of gp120 among the isolates of HIV-1 is another reason for the preference for this region. It has been assumed that the replacement of the sequence of seven amino acids in the V3 region had greater chances of leading to a viable recombinant than a mutation in another, more conservative, region of the HIV-1 genome. The recombinant protein gp120/R7V can be expressed in parallel in a suitable system for expression of a protein in order to obtain a large quantity of immunogen R7V.

The use of carrier proteins is not essential; it is possible to provide optionally for other carrier systems. "Carrier system" is intended to designate any component which makes it possible to lead to a unit generating an immune response against the peptide in question, or which makes it possible to protect the peptide from elimination, particularly from a rapid proteolysis.

The compositions according to the invention may also comprise components which increase the immunogenicity of the peptides and/or proteins, particularly immunity adjuvants, specific or otherwise, such as Freund's adjuvant, polysaccharides or equivalent compounds.

These are methods which are known in the vaccination field.

The compositions according to the invention can be used in any form compatible with the route of administration chosen, in particular the injectable route. However, it will be possible for the compositions according to the present invention to be used by other routes, particularly per os or by the aerosol route, to induce protection of the mucous membranes.

The present invention also relates to compositions intended to be administered in order to express in

situ the peptides and/or proteins described above.

In particular, the present invention relates to DNA expression cassettes which make it possible to express at least one cryptic epitope as defined above, and in particular the peptide having sequence 1 to 22 and/or the proteins having these peptides or proteins capable of coupling with the peptide in question as defined above or having equivalent sequences.

"Equivalent sequence" is intended to designate a sequence encoding an equivalent peptide as has been described above.

These DNA expression cassettes can, of course, be used either directly for expression in situ, or can be used to produce a peptide or protein which can be used as has been described above.

Vaccination systems using DNA sequences are known and are already widely described in the literature.

They are essentially systems allowing the expression of the antigenic protein in man, or the expression of the antigenic protein in a cell, which is then used for the vaccination; when the transformed cell is a host cell treated outside, the treatment is said to be ex vivo.

The expression systems may be highly varied; they may be in particular "naked DNA" type systems as are described in particular in the patents and patent applications of the company VICAL, WO 90/11092. In this case, the DNA encoding the peptide or protein comprising the peptide is injected as it is; this injection leads, in a number of cases, to the expression of the encoded protein.

The information contained in these documents is explicitly included in the present description by reference.

It will also be possible to use "naked DNA" systems, but comprising their own expression system, particularly in order to enhance the expression.

It will also be possible to use systems promoting the expression, either by integration, or by autonomous

replication, particularly of systems of the plasmid or viral type.

Among the systems for expression of a peptide sequence which may be mentioned, there should be mentioned the systems using poxviruses, adenoviruses, retroviruses and herpes-type viruses or other more recent systems such as polioviruses.

Among the vectors, vectors generating a humoral response and for the mucous membranes will preferably be used.

Other viruses can be used in order to obtain vaccines in particular:

- the adenoviruses as is described in N.R. Rabinovich et al., Science, 1994, 265, 1401-1404 and references cited;
- the rotaviruses as is described by Sue E. Crowford, in Journal of Virology, Sept. 1994, p. 5945-5952;
- the poxviruses, particularly the vaccinia virus, also animal poxviruses such as the canari pox as is described in the work by Paoletti and Moss;
- influenza virus as described in N.R. Rabinovich et al. (1994).

The technology which makes it possible to use the polioviruses as vaccination vector for various antigens is described particularly in Raul Andino et al., Science, 265, 1448-51.

This type of construct, which can be used in the context of the present invention, makes it possible to obtain vaccines which can be used by the oral route; to do this, the sequence encoding the peptide(s), optionally the carrier proteins, is cloned into a poliovirus, for example the attenuated Sabin virus; it is also possible to use a cocktail of viruses encoding various epitopes.

The use of plasmids or of viruses for the expression of proteins in the cells of a host, particularly a human host, is known and will not be explained in detail. The specific constructs obviously depend on the host, the epitope and the vector selected.

It is also possible to use cellular vaccines,

that is to say, for example as is proposed in the context of gene therapy, to collect cells from the patient, to transform them with vectors as described above and then to reimplant them in order to express the proteins in situ.

However, in the case of a vaccination, this method is not very convenient. It will be preferable to use cells which can be obtained in a large number, bacterial or yeast cells for example, which express the protein in question, for example at the surface, which, in some cases, increases the immunogenicity of the protein.

It is possible, for example, to use vaccines comprising, as expression system, Salmonella as is described in T.R. Fouts et al., Vaccine (1995) 13 in press; Tacket C.O. et al., Infect. Immun. (1992) 60, 536-541 and Hone et al., J. Chim. Invest. (1992) 90, 412-420 (for its evaluation in man as vaccinal support).

This type of vaccine involves the use of cells, particularly bacterial cells, producing the peptides according to the invention or certain strains of other vaccination vectors and described in Chad P Muller, Immunology Today. vol. 15 No. 20. 1994, p. 458-459.

The cells producing the peptides or proteins according to the invention can be used as they are, in particular when the proteins are expressed at the surface of the cells and when the cells are nontoxic and non-pathogenic (attenuated or killed strain), but can also be used to produce the peptides and/or proteins which will be used after purification.

Thus, it may be advantageous to obtain bacterial cells, but also yeasts or higher cells, animal, plant or insect cells in particular.

In the case of the present invention, it is possible to provide for the use of vaccines of plant origin using the technologies described particularly in C.J. Arntzel et al. in Vaccine 94.

The technologies allowing the expression of the peptides or proteins by cellular systems are known, as

well as the purification techniques.

As has already been mentioned, it is possible to use the compositions according to the invention with adjuvants enhancing the activity of the DNA sequences, particularly components constituting complexes with the DNA, such as cationic lipids or structures of the liposome or microparticle type.

The invention also relates to compositions containing antibodies against the peptides according to the invention or compositions containing sequences encoding antibodies directed against the peptides according to the invention.

Of course, the use of compositions based on antibodies requires that the latter are compatible with administration to a human being; they may be in particular antibodies humanized by known techniques or directly expressed in situ from the DNA sequence.

The present invention also relates to the use of the antibodies raised against the peptides of the invention and capable of neutralizing the HIV virus, in particular the present invention relates to anti-sera comprising this type of antibody or the antibodies obtained, for example by immunopurification, from the said sera.

The present invention also relates to a method of diagnosis, characterized in that the presence of an antibody directed against one of the epitopes according to the invention is detected in the serum of a patient.

This method can be carried out by any known method for identifying antibodies, particularly the ELISA and RIA methods and all the methods derived therefrom.

All these methods are preferably based on the attachment of the antibodies in question onto the antigenic peptides described above, followed by the visualization of this attachment. This diagnosis is of considerable interest; indeed, examples show that seropositive individuals in the case of HIV who have antibodies according to the invention do not, in a very large number of cases, progress, that is to say that they

do not develop AIDS. In this case, the prognosis is very favorable and it is possible to avoid expensive treatments. This is particularly true in the case of pregnancy where the presence of these antibodies in the mother  
5 (HIV +) would seem to lead to noninfection of the newborn.

The production of the compositions according to the present invention can be carried out by techniques which are known, synthesis of protein by the chemical  
10 route, synthesis of DNA by the chemical route or multiplication by PCR-type amplification. For the proteins, these can also be obtained by the recombinant route using appropriate syntheses.

The examples below will make it possible to  
15 demonstrate other characteristics and advantages of the present invention.

In the accompanying figures,

- Figures 1A and 1B represent ELISAs showing the reactivity of the serum of a rabbit immunized with R7V-KLH for various antigens,  
20
- Figure 2 represents the ELISA showing the reactivity of the antiserum of a rabbit immunized with in particular  $\beta 2m$ ,
- Figures 3A to 3D represent the ELISA between different antisera and selected peptides,  
25
- Figure 4 represents the ELISA for R7V with different anti- $\beta 2m$  antibodies,
- Figure 5 represents the ELISA for R7V-BSA and  $\beta 2m$  with anti- $\beta 2m$  antibodies and rabbit sera,
- 30 - Figures 6 to 13 represent diagrams showing the effect of the sera of different patients on the neutralization of different isolates of the HIV virus on MT4 and PBL.

#### EXAMPLE 1

35 This example makes it possible to demonstrate the immune response of rabbits against selected peptides coupled to a carrier protein.

The peptide antigen 7AA is coupled to KLH (Key-hole Limpet Hemocyanin) and injected into rabbits in the



presence of complete Freund's adjuvant.

The animals are immunized in the presence of complete Freund's adjuvant at D0, D14, D28, D42 and trial bleedings are carried out before immunization on days 35,  
5 49, 56 and 70.

The peptides used are: RV7-KLH, S7K-KLH and F7E-KLH

*Sul B5*  
10 The peptide R7V (RTPKIQV) was extended by 2 amino acids in order to allow the coupling. The structure used as immunogen is RTPKIQVGY.

The antibodies of the rabbits immunized 618 were measured by ELISA, where the peptide coupled to various carrier proteins was used at the bottom of the well (either coupled to KLH, to BSA (Bovine Serum Albumin) or  
15 MAP (Multiple Antigenic Peptide)).

The diagrams represent the results obtained at 2 dilutions d100 and d1000, that is to say a 1/100 and 1/1000 dilution of the sera, or at different times.

The ELISA method is applied in the following  
20 manner:

ELISA method

- 1) Attachment of the antigen onto a 96-well plate (Immulon IV-Dynatech)
  - . dilute the antigen in carbonate buffer pH 9.6
  - 25 → (Ag) final = 1 µg/ml
  - . distribute 100 µl/well, that is to say 100 ng/well
  - . incubate 2 h at 37°C or overnight at 4°C (humid atmosphere).
- 2) Washes
  - 30 . 5 washes with a solution of PBS/Tween 20 at 0.05%.
- 3) Saturation of the wells
  - . distribute 300 µl/well of a solution of PBS/horse (or bovine) serum at 10%
  - . incubate 1 h at 37°C (in a humid atmosphere).
- 35 4) Washes (identical to point 2)
- 5) Incubation with specific antisera
  - . dilute the serum (1/50, 1/100, 1/1000) with PBS-10% horse serum
  - . distribute 100 µl/well and incubate 1 h at 37°C

(in a humid atmosphere).

- 6) Washes (identical to point 2)
- 7) Incubation with the second antibody (sheep Ig's to human Ig's coupled to peroxidase)
  - 5 . dilute the second antibody 2/1000 in PBS/horse serum 10%
  - . distribute 100  $\mu$ l/well and incubate 1 h at 37°C (in a humid atmosphere).
- 8) Washes (identical to point 2)
- 10 9) Visualization with OPD
  - . dissolve 10 mg OPD in 25 ml of phosphocitrate buffer (0.1 M, pH 5.5)
  - . add at the last moment 10  $\mu$ l  $H_2O_2$
  - . distribute 100  $\mu$ l/well and incubate 30 min in the dark at room temperature (may be read at 405 nm)
  - 15 . stop the reaction with 50  $\mu$ l  $H_2SO_4$  12.5%.
- 10) Reading at 492 nm.

Figures 1A and 1B show results obtained with the rabbit 618 immunized with R7V-KLH.

- 20 An anti-R7V reactivity appears clearly as a differential between R7V-BSA and BSA compared with R7V-KLH and KLH where the anti-R7V reactivity is masked by the anti-KLH response of the serum. It should be noted that the anti-R7V reactivity is stronger at D68 than at
- 25 D132.

The specificity of the reaction is greater if the BSA protein is used.

Figure 2 again shows good recognition of the original protein,  $\beta$ 2m.

- 30 The antisera of immunized rabbits demonstrate a high reactivity with R7V-BSA as well as with the original peptides called P1, P4 and P9 which were used to select R7V, even though the reactivity with P1 is weaker (Figure 3A - 3D).

- 35 Figure 4 demonstrates that the recognition of R7V by B1G6 and B2G2.2 depends on the dose and that the recognition of C21.48 is not as good; accordingly, the mAbs B1G6 and B2G2.2 will preferably be used to select equivalent peptides.

These results demonstrate that the R7V epitope, coupled to BSA, is capable of generating a good immune response.

## EXAMPLE 2

### 5 Introduction of R7V into the V3 loop of HIV-1 LAV gp120 Construction of a recombinant provirus

The objective of this example is to introduce the R7V sequence into the third variable region V3 of the HIV-1 LAV gp120.

#### 10 Methods

Chimeric recombinant viruses were constructed by PCR-directed mutagenesis. Two constructs based on the R7V sequence and HIV-1 LAV were obtained, in which seven amino acids of the V3 region of gp120 have been replaced by the R7V sequence. The positions of the mutated sequences are shown in the following table:

HIV-1 LAV (V3)	NNNTRKSIRIQRGPGRAFVT		
R7V		RTPKIQV	(1) RPL
R7V		RTPKIQV	(2) PLG

20 The EcoRI<sub>5278</sub>-XhoI<sub>8401</sub> fragment of HIV-1 LAV cloned into the vector Bluescript was used as template for subsequent constructs. In the first stage, the DNA fragments flanked by primers containing the BglII restriction site at one end and the nucleotide sequence encoding R7V at the other end were synthesized for the RPL and PLG constructs by PCR amplification. The mutagenesis oligonucleotides used consisted of a (+) primer ACACCAAAGATACAAGTTGTTACAAATAGGAAAA and a (-) primer TTGTATCTTTGGTGTCTCTGGATCCGGATACTTT for the RPL construct

25 and of a (+) primer CGTACACCAAAAATCCAGGTCCAGAGAGGACCA and a (-) primer GATTTTGGTGTACGCGTATTGTTGTTGGGTCT for the PLG construct. In the second stage, two PCR products for each construct were mixed and amplified using the primers containing the BglII restriction sites. The RPL and PLG

30 fragments were cleaved by the enzyme BglII and inserted

35 into the vector Bluescript containing the EcoRI<sub>5278</sub>-XhoI<sub>8401</sub>

fragment of HIV-1 LAV, cleaved by BglII. In addition to the R7V sequence, the amplification primers contained modifications in the nucleotide sequence leading to the appearance of new BamHI and MluI restriction sites in the RPL and PLG constructs respectively, without additional modifications in the amino acid sequence. The new restriction sites were used to screen the mutated sequences. Finally, the EcoRI<sub>5278</sub>-XhoI<sub>8401</sub> fragments of HIV-1 LAV containing the RPL and PLG constructs were inserted into the plasmid pNL4-3 by homologous recombination using the EcoRI and XhoI restriction sites. The constructs were checked by restriction enzyme analysis.

#### Transfection of eukaryotic cells

The plasmid DNA of 200 ml of E. coli TG1 was extracted and purified by the Qiagen midiprep kit. The semiconfluent cultures of COS cells ( $= 4 \times 10^6$ ) were transfected with 7  $\mu$ g of plasmid by the calcium coprecipitation technique. The next day, the monolayers of cells were treated with glycerol and placed in coculture with a CEM cell line or with primary blood lymphocytes activated by PHA (PBL,  $10^6$  cells/ml) obtained from a healthy donor. The CEM or PBL cells were separated from the COS cells in monolayers two days later and cultured separately.

#### 25 Production of virus

1 ml of free cell supernatant obtained from the COS or PBL cells was ultracentrifuged and the virus sedimented was checked by the standard reverse transcriptase reaction. In some experiments, 100  $\mu$ l of cell supernatant was tested for the production of the p24gag protein.

Transfection of the COS cells and coculture with the  
CEM cells

	D. post-transf.	Reverse transcriptase activity (cpm/ml)		
		RPL 1	PLG 2	NL 4-3
5	5	7282	7730	45838
	9	3282	5302	326618
	13	382	630	ND
	16	200	300	ND

10  $4 \times 10^6$  COS cells were transfected per 7  $\mu$ g of plasmid by the calcium coprecipitation technique. The CEM cells were then added in an amount of  $4 \times 10^5$  cells/ml in a final volume of 5 ml. After two days of coculture, the CEM cells in suspension were separated from the COS cells in a monolayer. The reverse transcriptase activity in the CEM culture supernatants is given in cpm/ml.

15 Infection of the PBLs

D. post-inf.		Reverse transcriptase activity (cpm/ml)			
		RPL 1	PLG 2	NL4-3	
20	4	734	782	20008	
	7	202	216		
	10	262	282		
	14	454	262		
	17	204	138		
		RPL 1 + PBL PLG 2 + PBL			
25	1	350	336	276	636
	24	230	282	296	284
	27	588	510	620	980

2.5 × 10<sup>6</sup> PBLs were infected with the acellular supernatants of December 19, 1994 obtained after transfection (Table 1) in an amount of 5000 cpm/10<sup>6</sup> PBL. On day 17 post-infection, 2 × 10<sup>6</sup> newly isolated PBLs were added to 2 × 10<sup>6</sup> infected PBLs (RPL 1 + PBL, PLG 2 + PBL). The reverse transcriptase activity in the culture supernatants is given in cpm/ml.

Transfection of the COS cells and coculture with PBLs

10	D. post-transf.	Reverse transcriptase activity (cpm/ml)			
		PLG 2-25	PLG 2-30	PLG 2-95	NL 4-3
	3	2500	8400	3500	2150
	7	446	398	582	53000
	10	174	336	306	74000
	14	730	834	482	45778

15	D. post-transf.	R.T. activity (cpm/ml)	
		RPL 1	PLG 2
	3	20338	22000
	7	682	418
	11	552	466

4 × 10<sup>6</sup> COS cells were transfected with 7 µg of plasmid by the calcium coprecipitation technique. The PBL cells stimulated with PHA were then added in an amount of 10<sup>6</sup> cells/ml in a final volume of 5 ml. After two days of coculture, the PBLs in suspension were separated from the COS cells in a monolayer. The reverse transcriptase activity in the PBL culture supernatants is given in cpm/ml.

EXAMPLE 3

The aim of this example is to use the selected peptides to detect, in the serum of the patients, antibodies which are potentially inhibitors of HIV (anti-β<sub>2</sub>-microglobulin antibodies) and in particular to demon-



strate the presence of protective antibodies in the serum of patients who do not progress. "Patients who do not progress" or "NP" designate patients who have been seropositive for more than 10 years and who have not developed AIDS, in particular whose T4 cell level is normal.

#### Materials and methods

1/ The peptides used were synthesized and coupled to BSA by Néosystem (France).

10 2/ The sera of the patients are stored at -20° or -80°C before their use in Elisa.

3/ The second antibodies to human or rabbit Ig's were obtained from Amersham (France). OPD is obtained from Sigma (France).

#### 15 ELISA with the sera of seropositive patients

1/ Presence of anti-R7V antibodies in the serum of the patients (titre 1/100 and 1/1000).

20 2/ Of the 46 sera tested from people who do not progress (no viral replication in culture), 16 sera are positive for R7V (37%), 27 remain negative to 1/100 (63%) and 3 sera are impossible to determine (Table 1).

25 3/ Of the 46 patients who do not progress, 34 were tested for the detection of anti-peptide antibodies: R7V, P1, P4, P9. 14 sera are positive for at least one peptide (51.8%) and 13 remain negative to 1/100. Four sera could not be classified positive or negative (Table 2).

Table 1: ELISA R7V with NP sera

	NAME	NUMBER	R7V
	ARA GE	950	Negative
	ARG CH	150	Not determined
5	AUD PA	1509	Negative
	BAT AL	134	Negative
	BAR JE	342	Positive
	BER AL	704	Positive
	BER SE	1337	Negative
10	BES LA	287	Positive
	BEU PH	5.33	Negative
	BOR EM	194	Negative
	BOU NA	5.36	Positive
	BRE FR	20.2.95-5.32	Negative
15	CAB MI	573	Negative
	CAU BE	167/1113	Negative
	CHI OL	353A	Negative
	COU DA	1531	Negative
	DIB AN	872	Positive
20	DUR JE	937	Positive
	GAR AI	986	Negative
	GAS MA	549	Negative
	GUI JE	60	Positive
	GUI PI	26.1.95-2.9	Negative
25	HAN SO	169/5.31	Positive
	HOL CH	4.25	Negative
	IBE JU	6.39	Positive
	IMB PI	327	Not determined
	MAG HE	143	Negative
30	MAN GU	26.1.95-2.8	Negative
	MAN RO	89	Positive
	MAN XA	730	Negative
	MART DO	1412	Negative
	MAS SU	115	Negative
35	MEN JO	622/1382	Positive
	MON NA	1010	Negative
	NIC GE	294	Negative
	OUM NA	1386	Negative
	PAR FR	23.1.95-1.7	Negative
40	MEN JO	622/1382	Positive

5  
10

Table 2: ELISA for the peptides with the NP sera

	NAME	NUMBER	POSITIVE/ NEGATIVE	PEPTIDES			
				R7V	P1	P4	P9
5	BEU PH	5.33	Negative				
	BOU NA	5.36	Positive	Pos.	Pos.	Pos.	Neg.
	BRE FR	20.2.95-532	Positive	Neg.	Pos.	Pos.	Neg.
	CIF FR	6.38	Negative (?)				
	ETC MA	6.45	(?)				
10	GEM SA	6.40	(?)				
	GUI JE	60	Positive	Pos.	Pos.	Neg.	Neg.
	GUI PI	26.1.95-2.9	Negative				
	HAN SO	169/5.31	Positive	Pos.	Pos.	Neg.	Pos.
	HOL CH	4.25	Negative				
15	IBE JU	6.39	Positive	Pos.	Pos.	Neg.	Neg.
	LED DO	4.23	Positive	Neg.	Pos.	Neg.	Neg.
	MAN GU	26.1.95-2.8	Negative				
	MEN JO	622/1382/6.43	Positive	Pos.	Pos.	Pos.	Pos.
	MOR JE	5.37	Positive	Neg.	Neg.	Pos.	Neg.
20	PAR FR	23.1.95-1.7	Negative				
	PAT MA	166	Positive	Neg.	Neg.	Pos.	Neg.
	PIC CH	2.12	(?)				
	POI LI	3.14	Negative				
	PUJ MA	23.1.95-1.2	Negative				
25	QUI AL	23.1.95-1.5	Negative				
	RIO EM	3.16	Negative				
	RIS HE	2.10	Negative				
	ROY CH	5.35	Positive	Pos. (?)	Pos.	Neg.	Neg.
	SAL YA	13/3.95	Negative				
30	SAN NA	2.11	Negative				
	SAP MA	4.21	(?)				
	SAU CH	171/4.27	Positive	Pos. Pos. (?)	Pos.		Pos.
	SEN AN	4.28	Positive	Neg.	Pos.	Neg.	Neg.
	TEM ST	5.34	Positive (?)	Pos.	(?)	(?)	(?)
	ZUM AM	333	Positive	Pos.	(?)	(?)	(?)

(?) Not determined

#### EXAMPLE 4

The following trials made it possible to detect antibodies neutralizing various HIV isolates, particularly BRU and NDK, in patients who do not progress, the same patients having anti-R7V antibodies. This makes it possible to show a good correlation between the neutralizing and protective character against HIV of the antibodies generated by the treatments according to the invention.

#### 10 Materials and methods

##### Culture of the MT4 cells

The MT4 cells are immortalized cells (CD4 +) which are very sensitive to the cytopathogenic effect of HIV-1, originally derived from a T leukaemia in adults. The cells are cultured in the presence of RPMI medium supplemented with 10% of foetal calf serum, 1% of glutamine and 1% of antibiotic.

##### Culture of the PBLs

The lymphocytes are stimulated for 3 days with phytohemagglutinin P (PHA P) in complete RPMI medium comprising 10% of foetal calf serum, 1% of glutamine, 1% of antibiotic, 2 µg/ml of polybren, 20 IU/ml of interleukin 2 (IL-2). The cells are then washed and cultured in an amount of 10<sup>6</sup> cells/ml in complete RPMI medium.

#### Neutralization tests

The sera are deplementized and filtered before they are used in the tests.

##### Neutralization on MT4

The sera are diluted in 24-well plates (Costar) in a total volume of 0.8 ml. the HIV-1 BRU viruses (100 µl of a 10<sup>-1</sup> dilution of the stock solution) or HIV-1 NDK viruses (100 µl of a 10<sup>-3</sup> dilution of the stock solution) are added and the mixture is incubated for 1 h 30 min at 37°C and 5% CO<sub>2</sub>. The cells are then distributed

in an amount of 200  $\mu$ l/well and  $1.5 \times 10^6$  cells/ml. Three days after the infection, the cultures are diluted (1/3) with 10% RPMI medium. The infection of the cells with the HIV-1 viruses is monitored every day under a microscope by observing the formation of syncytia (multinucleated giant cells). The neutralization of the viruses with the different sera is defined by the absence (-) of syncytia or very few syncytia (+/-) compared with the formation of syncytia which is induced by the (+) prototype HIVs.

10 Neutralization on PBL

The sera (50  $\mu$ l) are mixed with the HIV-1 BRU viruses (50  $\mu$ l of a  $2 \times 10^{-1}$  dilution of the stock solution) in 96-well plates and placed for 1 h 30 min at 37°C and 5% CO<sub>2</sub>. The mixture is then added to  $10^6$  PBLs in 24-well plates (Costar) and the culture is maintained for 3 days at 37°C and 5% CO<sub>2</sub>. The cells are then washed and cultured in an amount of  $10^6$  cells/ml in 25 cm<sup>2</sup> culture flasks. The production of virus is monitored every 3 or 4 days by assaying the "Reverse Transcriptase" enzymatic activity.

Assay of the "Reverse Transcriptase" (RT) activity

One ml of centrifuged culture supernatant (1500 rpm, RT, 10 min) is concentrated 100 fold by ultracentrifugation (95000 rpm, 4°C, 5 min) on a TL 100 rotor (Beckman). The pellet obtained is taken up in 10  $\mu$ l of NTE buffer - 0.1% Triton X100. The enzymatic reaction is carried out in 50  $\mu$ l of the following reaction mixture: 50 mM Tris pH 7.8; 20 mM MgCl<sub>2</sub>; 20 mM KCl; 2 mM dithiothreitol; Oligo dT 12-18 0.25 OD/ml; poly rA 0.25 OD/ml and 2.5  $\mu$ Ci of <sup>3</sup>HdTTP. After incubating for 1 h at 37°C, the reaction products are precipitated with 20% trichloroacetic acid, filtered on Millipore membranes and the  $\beta$  radioactivity is measured. The results are expressed in CMP/ml.

35 Report for the neutralization experiments

Antibodies directed against the peptide R7V were



detected in the sera of HIV + patients by means of a specific ELISA developed by the Applicant. A search was made in these sera for the existence of a neutralizing activity directed against the two prototype virus strains  
5 HIV-1 BRU and NDK. Two neutralization tests were carried out, one on an MT4 cell line (followed by the formation of syncytia) and the other on healthy peripheral blood lymphocytes, PBL (followed by the "Reverse Transcriptase" enzymatic activity).

10 Results obtained on MT4

Of the 13 patients tested, a neutralizing serum activity was detected for 6 of them (Tables 3 to 5):

- two sera neutralize HIV-1 NDK:  
ZUM AM (ELISA positive)  
15 COC PH (ELISA negative)
- two sera neutralize HIV-1 BRU:  
MEC EV (ELISA positive)  
OUA VE (ELISA negative)
- two sera neutralize HIV-1 BRU and HIV-1 NDK:  
20 SAU CH (ELISA positive)  
BUB JE (ELISA positive)

Results obtained on PBL

The experiment was carried out with the sera of MEC EV, SAU CH and BUB JE (1/50) as well as with a serum  
25 from a seronegative individual. No neutralizing activity was detected for the SAU CH serum as well as for the seronegative serum. On the other hand, a neutralizing activity against the two prototype viruses HIV-1 BRU and NDK was detected for the sera MEC EV and BUB JE (Figures  
30 6 to 13).

EXAMPLE 5

Method which makes it possible to detect equivalent peptides

Effect of selected peptides on the neutralization of HIV-

5 1 NDK by anti-B1G6  $\beta$ 2 monoclonal antibodies

Protocol

10 The peptides at a concentration of 100  $\mu$ g/ml or 50  $\mu$ g/ml (40  $\mu$ l or 20  $\mu$ l of the stock solution and 5 mg/ml) are preincubated with 5  $\mu$ g/ml of B1G6 (10  $\mu$ l of a stock solution at 1 mg/ml) in a total volume of 110  $\mu$ l for 2 hours, in tubes on a water bath at 37°C, with gentle stirring. Next, HIV-1 NDK is added (100  $\mu$ l of a  $2 \times 10^{-4}$  dilution of a stock solution and the tubes are incubated for 1 hour at 37°C on a water bath. The tubes  
15 are then separated into two and each 100  $\mu$ l is added to  $10^6$  PBLs on a 24-well plate. The cells are cultured for 3 days at 37°C under an atmosphere with 5% CO<sub>2</sub>. On day 3, the cells are washed, placed in culture and propagated for at least 20-25 days in a 25 cm<sup>3</sup> round-bottomed flask.  
20 The production of virus is monitored every 3 or 4 days by the assay of reverse transcriptase (RT).

Results

25 The peptides R7V and F7E can cancel the neutralizing effect of the monoclonal antibody B1G6 on the productin of HIV-1 NDK by the PBLs. The sequence of the R7V peptide was modified and among the 6 new peptides (185, 186, 187, 188, 189, 190), 3 lost the canceling effect of R7V: peptides 185, 189 and 190).

Table 3

NDK												
Day / Post-infection												
	D4			D5			D6			D7		
	1/25	1/50	1/100	1/25	1/50	1/100	1/25	1/50	1/100	1/25	1/50	1/100
HIV+												
ZUM AM	-	-	-	-	-	-	-	-	-	-	-	+
HAN SO	-	-	-	+	-	-	+	+/-	+/-	+	+	+
SAU CH	-	-	-	+	+	+	+	+	+	+	+	+
MEN JO	-	-	-	+/-	-	+/-	+	+/-	+	+	+/-	+
PAT MA	+/-	-	-	+/-	+	-	+/-	+/-	-	+/-	+/-	-
COC PH	-	-	-	-	-	+	-	-	+	-	-	+
HIV-												
SER C	-	-	-	+	+	+	+	+	+	+	+	+
DOU S	-	-	-	+	+	+	+	+	+	+	+	+
AUB V	-	-	-	+	+	+	+	+	+	+	+	+
NDK 10''												
		+/-		+			+			+		

Table 4a

NDK										
Day / Post-infection										
	D4		D5		D6		D7			
	1/50	1/100	1/50	1/100	1/50	1/100	1/50	1/100	1/50	1/100
HIV+										
ZUM AM	-	+/-	+	+	+	+	+	+	+	+
HAN SO	+	+/-	+	+	+	+	+	+	+	+
SAU CH	+	+	+	+	+	+	+	+	+	+
MEN JO	+	+	+	+	+	+	+	+	+	+
MEC EV	+/-	-	+/-	+	+	+	+	+	+	+
PAT MA	+/-	+/-	+/-	+	+	+	+	+	+	+
COC PH	+/-	-	+/-	-	+	+	+	+	+	+
HIV-										
AUB V	+	+/-	+	+	+	+	+	+	+	+
NDK 10 <sup>4</sup>		+		+			+			+

Table 4b

BRU									
Day / Post-infection									
	D4		D5		D6		D7		
	1/50	1/100	1/50	1/100	1/50	1/100	1/50	1/100	
HIV+									
ZUM AM	+/ -	+/ -	+/ -	+	+	+	+	+	+
HAN SO	+/ -	+/ -	+/ -	+	+	+	+	+	+
SAU CH	+	+	+	+	+	+	+	+	+
MEN JO	-	+/ -	+/ -	+/ -	+	+	+	+	+
MEC EV	-	-	-	-	-	+	-	+	+
PAT MA	+/ -	-	+/ -	+/ -	+	+	+	+	+
COC PH	-	+/ -	+/ -	+	+	+	+	+	+
HIV-									
AUB V	+	+	+	+	+	+	+	+	+
BRU 10 <sup>-2</sup>		+/ -		+		+		+	

Table 5a

NDK												
Day / Post-infection												
	D4			D5			D6			D7		
	1/25	1/50	1/100	1/25	1/50	1/100	1/25	1/50	1/100	1/25	1/50	1/100
HIV+												
ZUM AM	-	+/-	+/-	-	+/-	+	-	+	+	-	+	+
MEC EV	-	-	-	+	+/-	-	+	+	+	+	+	+
SAU CH	-	+/-	+/-	-	-	-	-	-	+/-	-	-	+
OUA VE	-	-	+/-	-	+/-	+	+/-	+	+	+	+	+
QUI AL	+/-	+/-	+/-	+	+	+	+	+	+	+	+	+
BUB JE	+/-	-	+/-	-	+/-	+/-	-	+	+	-	+	+
PUJ MA	+/-	+/-	+/-	+/-	+/-	+	+/-	+/-	+	+/-	+	+
SEN AN	+/-	+/-	+	+	+	+	+	+	+	+	+	+
RIO EM	+/-	+/-	+/-	+	+	+	+	+	+	+	+	+
HIV-												
AUB V	+	+	+	+	+	+	+	+	+	+	+	+
NDK 10**		+/-			+			+			+	

Table 5b

BRU												
Day / Post-infection												
	D4			D5			D6			D7		
	1/25	1/50	1/100	1/25	1/50	1/100	1/25	1/50	1/100	1/25	1/50	1/100
HIV+												
ZUM AM	-	+/-	+	+	+	+	+	+	+	+	+	+
MEC EV	-	-	-	-	-	-	-	-	-	-	-	+/-
SAU CH	-	-	-	-	-	-	-	-	+	-	-	+
OUA VE	-	-	-	-	-	+/-	-	+	+	-	+	+
QUI AL	-	-	-	-	-	+	+	+/-	+	+	+	+
BUB JE	-	-	-	-	-	-	-	+/-	+/-	-	+	+
PUJ MA	-	-	+/-	+/-	+	+	+	+	+	+	+	+
SEN AN	-	-	+/-	+/-	+	+/-	+	+	+	+	+	+
RIO EM	+/-	+/-	+/-	+	+	+	+	+	+	+	+	+
HIV-												
AUB V	+	+	+/-	+	+	+	+	+	+	+	+	+
BRU 10 <sup>-2</sup>		+	1.0	+	+		+	+		+	+	



**EXAMPLE 6**

**Correlation between the presence of anti-R7V antibodies and the progression of the disease**

5 Serum samples from 90 patients infected with HIV are used. They are distributed as follows: 28 patients who have been asymptomatic for more than 3 years, 24 long-term survivors and 38 patients suffering from Aids. A control group consisting of 69 seronegative volunteer donors was obtained from the blood bank.

10 The lymphocytes were counted by indirect immunofluorescence and analyzed by Epic Profile (Coultronics, Margency, France). The  $\beta$ 2m serum levels were measured by immunodiffusion (El Nanorid Kit). The p24 antigen levels were tested by the Coulter p24 detection  
15 kit (Coultronics, Margency, France).

The serum concentrations of anti-R7V antibodies are detected by ELISA. The results are expressed as concentration of B1G6 monoclonal antibody equivalent in  $\mu$ g/ml.

20 **Neutralization trial**

The human sera are deplementized and diluted up to 200  $\mu$ g/ml or 100  $\mu$ g/ml of B1G6 equivalent. 50  $\mu$ l of HIV containing 100 TCID<sub>50</sub> are preincubated with 50  $\mu$ l of dilute serum (total volume 100  $\mu$ l) in a 96-well plate at  
25 37°C and 5% CO<sub>2</sub> for 90 min. The reaction mixture containing the viruses and the serum is diluted twice after addition of  $8 \times 10^4$  MT4 cells (final dilution of the sera from 1/120 to 1/20) and three times again three days after the infection. The fusogenic effect of HIV in  
30 the MT4 lines, that is to say the formation of syncytia as an indicator of infection by the virus, is monitored for 7 days in the culture wells. The Reverse Transcriptase activity is measured in 400  $\mu$ l of supernatant free of cell, 7 days after the infection.

35 The mean value of the anti-R7V antibody levels is calculated for each patient and for each group. The sera of people infected with HIV contain anti-R7V antibodies and the HIV seropositive sera show significantly higher

concentrations of anti-R7V antibodies than seronegative sera. The anti-R7V antibody levels, expressed as B1G6 equivalent, range from 35 to 2558  $\mu\text{g/ml}$  ( $n=90$ ) and from 27 to 1790  $\mu\text{g/ml}$  ( $n=69$ ), respectively, in the groups infected with HIV and in the groups not infected with HIV.

The group with the HIV patients was then classified into three categories according to their clinical status: the group with those who do not progress (NP) consisting of the patients who have been seropositive for HIV for a long period and have been monitored in the laboratory for more than 3 years without Aids symptoms; the group with long-term survivors (LTS) consists of people who have had Aids for a long period, and finally a group which progresses consists of people suffering from Aids with a bad prognosis.

The anti-R7V antibodies are significantly increased in the asymptomatic group (from 91 to 2558  $\mu\text{g/ml}$ ) compared with the group which progresses (from 35 to 630  $\mu\text{g/ml}$ ) ( $p=0.001$ ) whereas no significant difference is observed compared with the LTS group (from 59 to 1864  $\mu\text{g/ml}$ ). Likewise, the LTS group has higher anti-R7V levels than the group which progresses ( $p=0.004$ ). Compared with the healthy subjects, there is no difference in the anti-R7V antibody level in the group which progresses.

In the group which progresses, a clear distinction can be made according to the anti-R7V antibody level between the subjects who die shortly after their last visit to the laboratory (from 35 to 508  $\mu\text{g/ml}$ ,  $n=23$ ) and those still alive but ill (from 77 to 586  $\mu\text{g/ml}$ ,  $n=14$ ) ( $p<0.03$ ).

A longitudinal follow-up study was not able to establish a correlation between the anti-R7V antibody levels and other biological parameters such as total lymphocyte count, CD4 and CD8 cells, p24 and  $\beta 2\text{m}$  in circulation.

It appears that the R7V level is stable over time in the NP patients, whereas it fluctuates in the LTS

patients.

In order to link the ELISA test with a biological activity of the patient's serum, a neutralization test was carried out with two nonrelated viruses, the HIV-1 LAV strain and the highly cytopathogenic HIV-1 NDK strain, on indicator MT4 cells. The serum dilutions were adjusted in order to obtain 5  $\mu$ g of B1G6 equivalent in the neutralization mixture. This concentration was defined as optimum for neutralizing the infection by the B1G6 antibodies. As seen in Table 5, 17 of the 18 sera selected prevent the infection of MT4 cells both by NDK and by LAV. To obtain 5  $\mu$ g of B1G6 equivalent in culture, 13 of the 18 sera tested required a dilution less than 1/50. In order to eliminate nonspecific activities due to possible serum components, these sera with a low B1G6 equivalent level were diluted 1/100 and used in the neutralization trial. The quantity of B1G6 equivalent in culture was then less than 5  $\mu$ g (from 2.5  $\mu$ g/ml to 0.3  $\mu$ g/ml). Nine of the 14 sera (64%) still neutralized both HIV strains, LAV and NDK, at a 1/100 dilution. Three sera from healthy donors used as controls show no neutralizing activity.

Table 5

25	Dilutions of the serum	:	Number of sera which neu-
	at 5 $\mu$ g of B1G6 equiv-	:	tralize the two strains
	alent in the trial	:	of HIV/total sera tested
<hr/>			
	dilution $\geq$ 1/50	:	5/5
	1/50 > dilution $\geq$ 1/20	:	10/11
	dilution < 1/20	:	2/2
<hr/>			
30	TOTAL	:	17/18
<hr/>			

SEQUENCE LISTING

Information for SEQ ID No. 1

TYPE: amino acid

LENGTH: 15 amino acids

5 TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 1

Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr-Ser-Arg-His-Pro-Ala

Information for SEQ ID No. 2

10 TYPE: amino acid

LENGTH: 15 amino acids

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 2

15 Phe-His-Pro-Ser-Asp-Ile-Glu-Val-Asp-Leu-Leu-Lys-Asp-Gly-Glu

Information for SEQ ID No. 3

TYPE: amino acid

LENGTH: 15 amino acids

TOPOLOGY: linear

20 SEQUENCE DESCRIPTION: SEQ ID No. 3

Ala-Cys-Arg-Val-Asn-His-Val-Thr-Leu-Ser-Gln-Pro-Lys-Ile-Val

Information for SEQ ID No. 4

TYPE: amino acid

25 LENGTH: 7 amino acids

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 4

Arg-Thr-Pro-Lys-Ile-Gln-Val

Information for SEQ ID No. 5

5 TYPE: amino acid  
LENGTH: 7 amino acids  
TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 5

Ser-Gln-Pro-Lys-Ile-Val-Lys

10 Information for SEQ ID No. 6

TYPE: amino acid  
LENGTH: 7 amino acids  
TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 6

15 Phe-His-Pro-Ser-Asp-Ile-Glu

::

Information for SEQ ID No. 7

TYPE: amino acid  
LENGTH: 10 amino acids  
TOPOLOGY: linear

20 SEQUENCE DESCRIPTION: SEQ ID No. 7

Thr-Leu-Ser-Arg-Thr-Pro-Lys-Ile-Gln-Val

Information for SEQ ID No. 8

TYPE: amino acid

LENGTH: 10 amino acids

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 8

Ile-Tyr-Leu-Thr-Gln-Pro-Lys-Ile-Lys-Val

5 Information for SEQ ID No. 9

TYPE: amino acid

LENGTH: 10 amino acids

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 9

10 Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr

Information for SEQ ID No. 10

TYPE: amino acid

LENGTH: 10 amino acids

TOPOLOGY: linear

15 SEQUENCE DESCRIPTION: SEQ ID No. 10

Thr-Leu-Ser-Gln-Pro-Lys-Ile-Val-Lys-Asn ::

Information for SEQ ID No. 11

TYPE: amino acid

LENGTH: 10 amino acids

20 TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 11

Ile-Gln-Arg-Thr-Pro-Gln-Ile-Val-Lys-Trp

Information for SEQ ID No. 12

TYPE: amino acid  
LENGTH: 10 amino acids  
TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 12

5 Ile-Gln-Arg-Thr-Pro-Asn-Ile-Val-Lys-Trp

Information for SEQ ID No. 13

TYPE: amino acid  
LENGTH: 8 amino acids  
TOPOLOGY: linear

10 SEQUENCE DESCRIPTION: SEQ ID No. 13

Cys-Tyr-Asn-Pro-Ser-Asp-Ile-Glu

Information for SEQ ID No. 14

15 TYPE: amino acid  
LENGTH: 7 amino acids  
TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 14

Tyr-Cys-Asn-Pro-Glu-Ser-Thr

Information for SEQ ID No. 15

20 TYPE: amino acid  
LENGTH: 8 amino acids  
TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 15

Asn-Phe-Leu-Asn-Cys-Tyr-Val-Ser



Information for SEQ ID No. 16

TYPE: amino acid

LENGTH: 9 amino acids

TOPOLOGY: linear

5 SEQUENCE DESCRIPTION: SEQ ID No. 16

Leu-Asn-Cys-Tyr-Val-Ser-Pro-Ser-Asp

Information for SEQ ID No. 17

TYPE: amino acid

LENGTH: 7 amino acids

10 TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 17

Lys-Thr-Pro-Gln-Ile-Gln-Val

Information for SEQ ID No. 18

TYPE: amino acid

15 LENGTH: 7 amino acids

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 18

Phe-His-Pro-Pro-Gln-Ile-Glu

Information for SEQ ID No. 19

20 TYPE: amino acid

LENGTH: 7 amino acids

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 19

Phe-His-Pro-Pro-His-Ile-Glu

Information for SEQ ID No. 20

TYPE: amino acid  
LENGTH: 7 amino acids  
TOPOLOGY: linear

5 SEQUENCE DESCRIPTION: SEQ ID No. 20

Ala-Glu-Pro-Lys-Thr-Val-Tyr

Information for SEQ ID No. 21

10 TYPE: amino acid  
LENGTH: 7 amino acids  
TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 21

Ser-Gln-Pro-Lys-Thr-Val-Tyr

Information for SEQ ID No. 22

15 TYPE: amino acid  
LENGTH: 10 amino acids  
TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID No. 22

Ile-Leu-Ser-Arg-Thr-Pro-Lys-Ile-Gln-Val

LEGEND TO THE FIGURES

## LEGEND TO FIGURE 1A

<input checked="" type="checkbox"/>	J63	d100
<input checked="" type="checkbox"/>	J132	d100

## LEGEND TO FIGURE 1B

<input checked="" type="checkbox"/>	J63	d100
<input checked="" type="checkbox"/>	J132	d100

## 5 LEGEND TO FIGURE 2

ELISA with rabbit antiserum for R7V or  $\beta$ 2m  
 rabbit 618 (immunization with R7V-KLH) serum dilution  
 1/100

<input checked="" type="checkbox"/>	IMMUNE SERUM
<input checked="" type="checkbox"/>	J63 pi
<input checked="" type="checkbox"/>	J132 pi

## LEGEND TO FIGURE 3A

## 10 ELISA with rabbit antiserum on wells coated with peptide

<input checked="" type="checkbox"/>	rabbit 618 immunized with R7V
<input checked="" type="checkbox"/>	rabbit 621 immunized with F7E
<input checked="" type="checkbox"/>	rabbit 624 immunized with S7K

## LEGEND TO FIGURE 3B

ELISA with sera of immunized rabbits

- |                                     |            |
|-------------------------------------|------------|
| <input type="checkbox"/>            | rabbit 618 |
| <input checked="" type="checkbox"/> | rabbit 619 |
| <input type="checkbox"/>            | rabbit 620 |

## LEGEND TO FIGURE 3C

5 ELISA with the sera of immunized rabbits

- |                                     |            |
|-------------------------------------|------------|
| <input type="checkbox"/>            | rabbit 621 |
| <input checked="" type="checkbox"/> | rabbit 622 |
| <input checked="" type="checkbox"/> | rabbit 623 |

## LEGEND TO FIGURE 3D

ELISA with sera of immunized rabbits

- |                                     |            |
|-------------------------------------|------------|
| <input type="checkbox"/>            | rabbit 624 |
| <input checked="" type="checkbox"/> | rabbit 625 |
| <input type="checkbox"/>            | rabbit 626 |

## LEGEND TO FIGURE 4

ELISA with B1G6, C21.43, B2G2.2 mAb for R7V

- |                                     |        |
|-------------------------------------|--------|
| <input type="checkbox"/>            | B1G6   |
| <input checked="" type="checkbox"/> | C21.48 |
| <input checked="" type="checkbox"/> | B2G2.2 |

## LEGEND TO FIGURE 5

ELISA FOR R7V-BSA OR  $\beta$ 2

<input type="checkbox"/>	R7V
<input checked="" type="checkbox"/>	BETA2m

## LEGEND TO FIGURE 6

5 NEUTRALIZATION OF HIV-1 BRU-1 WITH THE SERUM MEC EV  
(1/50) ON PBL

10

—△—	MEC EV 50
—△—	MEC EV 50'
—★—	BRU
—★—	BRU'

## LEGEND TO FIGURE 7

NEUTRALIZATION OF HIV-1 BRU-1 WITH THE SERUM OF BUB JE  
(1/50) ON PBL

15

—△—	BUB JE 50
—△—	BUB JE 50'
—★—	BRU
—★—	BRU'

## LEGEND TO FIGURE 8

EFFECT OF THE SERUM SAU CH (1/50) ON THE PRODUCTION OF  
HIV-1 BRU-1 ON PBL

- 5
- △— SAU CH 50
  - △— SAU CH 50'
  - ★— BRU
  - ★— BRU'

## LEGEND TO FIGURE 9

10 EFFECT OF A SERUM OF AN HIV- PATIENT ON THE PRODUCTION OF  
HIV-1 ON PBL

- 15
- △— SN5
  - △— SN5'
  - ★— BRU
  - ★— BRU'

## LEGEND TO FIGURE 10

NEUTRALIZATION OF HIV-1 NDK WITH THE SERUM MEC EV (1/50)  
ON PBL

- 20
- △— MEC EV 50
  - △— MEC EV 50'
  - ★— NDK 5-4

## LEGEND TO FIGURE 11

NEUTRALIZATION OF HIV-1 NDK WITH THE SERUM BUB JE (1/50)  
ON PBL

- 5      —△— BUB JE 50  
       —△— BUB JE 50'  
       —★— NDK 5-4

## LEGEND TO FIGURE 12

10      EFFECT OF THE SERUM SAU CH (1/50) ON THE PRODUCTION OF  
       HIV-1 NDK ON PBL

- △— SAU CH 50  
       —△— SAU CH 50'  
       —★— NDK 5-4

## LEGEND TO FIGURE 13

15      EFFECT OF A SERUM OF AN HIV- PATIENT ON THE PRODUCTION OF  
       HIV-1 NDK ON PBL

- △— SN5 50  
       —△— SN5 50'  
       —★— NDK 5-4